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(54) Title: CLEAVAGE OF FUSION PROTEINS USING GRANZYME B PROTEASE

(57) Abstract: A method for the preparation of a polypeptide of interest in authentic form by enzymatic cleavage of fusion proteins using Granzyme B protease (EC 3.4.21.79). There is also provided fusion proteins comprising a polypeptide of interest and a fusion partner, wherein the junction region between the polypeptide of interest and the fusion partner comprises a Granzyme B protease cleavage site adjacent to the polypeptide of interest, and a human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.



**WO 2004/094478 A3**

## INTERNATIONAL SEARCH REPORT

Int lonal Application No  
PCT/DK2004/000282

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C07K19/00 C12N9/64

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, Sequence Search, WPI Data, PAJ, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HARRIS J L ET AL: "Definition and redesign of the extended substrate specificity of granzyme B." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 16 OCT 1998, vol. 273, no. 42, 16 October 1998 (1998-10-16), pages 27364-27373, XP002296677 ISSN: 0021-9258 cited in the application abstract  ----- -/--	1-39



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*G\* document member of the same patent family

Date of the actual completion of the international search

12 November 2004

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02.12.04

Name and mailing address of the ISA

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SUN JIURU ET AL: "Importance of the P4' residue in human granzyme B inhibitors and substrates revealed by scanning mutagenesis of the proteinase inhibitor 9 reactive center loop"</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 18, 4 May 2001 (2001-05-04), pages 15177-15184, XP002296678            ISSN: 0021-9258            cited in the application            abstract</p>	1-39
A	<p>WO 03/010204 A (KIM JEONG-MIN ; CHO TAE-HOON (KR); LEE WOO-JONG (KR); PARK HEUNG-BOK ()) 6 February 2003 (2003-02-06)            cited in the application            abstract            page 3, lines 8-32            page 5, line 29</p>	1-39
A	<p>US 6 010 883 A (THOEGERSEN HANS CHRISTIAN ET AL) 4 January 2000 (2000-01-04)            cited in the application            abstract            column 2, lines 1-40</p>	1-39
A	<p>SUN JIURU ET AL: "Expression and purification of recombinant human granzyme B from Pichia pastoris"</p> <p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 261, no. 2, 2 August 1999 (1999-08-02), pages 251-255, XP002296679            ISSN: 0006-291X            abstract</p>	1-39
A	<p>ROTONDA J ET AL: "THE THREE-DIMENSIONAL STRUCTURE OF HUMAN GRANZYME B COMPARED TO CASPASE-3, KEY MEDIATORS OF CELL DEATH WITH CLEAVAGE SPECIFICITY FOR ASPARTIC ACID IN P1"</p> <p>MOLECULAR CELL, CELL PRESS, CAMBRIDGE, MA, US, vol. 8, no. 4, April 2001 (2001-04), pages 357-368, XP001183179            ISSN: 1097-2765            abstract</p>	1-39
A	<p>TRAPANI J A: "Granzymes: a family of lymphocyte granule serine proteases."</p> <p>GENOME BIOLOGY 2001, vol. 2, no. 12, 2001, page REVIEWS3014, XP002305250            ISSN: 1465-6914            abstract</p>	1-39

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK2004/000282

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

information on patent family members

In: tional Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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